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PRINCIPAL INVESTIGATOR: Lonnie D. Shea, Ph.D.

CONTRACTING ORGANIZATION: Northwestern University
                          Evanston, IL 60208

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Lonnie D. Shea, Ph.D.

E-Mail: l-shea@northwestern.edu

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14. ABSTRACT

A system for the large scale, dynamic quantification of transcription factor activity was applied to investigate the mechanism of action for PARP inhibitors. Of the 34 TFs investigated, 15 had differential activity in the presence of the PARP inhibitor olaparib. A few of the TF had differential activity in BRCA1-wild type and BRCA1-null cells. This differential activity may underlie why some patients respond whereas others are resistant. In addition, five PARP-1 inhibitor resistant clones were established from BRCA1-null type cells. Cell array analysis from these clones will provide us valuable insights into addressing the occurrence of resistance. We are continuing to investigate these factors to identify whether targeting these additional pathways can increase the efficacy or reduce resistance to PARP inhibitors.

15. SUBJECT TERMS
Olaparib, PARP inhibitors, transcription factor, cell array

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Identification of a PARP Inhibitor Sensitivity Signature in Breast Cancer using A Novel Transcription Factor Activity Array

PIs: Shea, Cryns, Jeruss

INTRODUCTION: Breast cancer is the leading cause of cancer and the second leading cause of cancer mortality in women. Familial breast cancer accounts for approximately 5 to 10% of breast cancer cases. Germline mutations of the breast tumor suppressor genes BRCA1 and BRCA2 have been found to contribute to most of the familial breast cancer cases. Inhibition of PARP1, which is involved in the base excision repair (BER) pathway, leads to the persistence of single-strand DNA lesions [1]. During DNA replication, these DNA nicks can degenerate to form double-strand breaks. When PARP-1 is blocked, backup DNA recombination mechanisms, such as homologous recombination, normally become activated. As BRCA1 and BRCA2 proteins are critical players in the homologous repair pathway, the combination of PARP inhibitors in BRCA-defective cancer cells is thought to lead to unsustainable genetic damage and cell death (synthetic lethal effect). In addition, normal tissue, which contains at least one functional allele of BRCA1 or BRCA2 with which to repair its DNA, should be spared when exposed to a PARP inhibitor. PARP inhibitors have shown preclinical efficacy in tumors with homologous DNA repair defects such as those arising in BRCA1 or BRCA2 mutation carriers with breast cancer and ovarian cancer [2-4]. Clinical trials have been also performed. Olaparib (AZD2281) is a small-molecule and potent oral PARP inhibitor [5-9]. In a phase 1 study, responses were reported in an expanded cohort of BRCA1 or BRCA2 mutation carriers with ovarian cancer [10, 11]. Subsequent phase 2 studies of BRCA1 or BRCA2 mutation carriers have confirmed the activity of olaparib monotherapy with objective response rates of 41% (11 of 27) in patients with advanced breast cancer and 33% (11 of 33) in those with ovarian cancer [12]. However, it was also reported that iniparib (BSI-201), currently the most clinically advanced PARP inhibitor [13-15], failed to prolong survival in the first phase 3 trial of metastatic, triple-negative breast cancer (TNBC) patients despite promising phase 2 trial results [16]. Cancer treatments have traditionally been investigated without complete understanding of the mechanisms underlying response and resistance. Therefore, investigating the mechanism of PARP1 inhibition to induced cell death and development of resistance during treatment may identify strategies to maximize the drug efficacy, develop strategies to overcome resistance, and may identify patients that are most likely to respond to PARP1 inhibitors. Initial studies implicated PARP1 to be involved in many crucial biological functions including DNA repair, replication, recombination, apoptosis and cancer. Evidence began to emerge from later studies that PARP1 also has a profound role in transcription. PARP1 was identified among the constituents of the positive cofactor-1 complex. Several independent studies revealed its potent effect on the transcription factors (TF), AP1, AP2, p53, NF-κB, B-Myb, TEF-1/Max [17-22], but the mechanisms and the scope of its impact is largely unknown.

We performed large-scale measurements of TF activity, which are determinants of the cellular response or resistance to PARP1 inhibition, as a means to identify the cellular response to PARP1 inhibitors in breast cancer cell lines with and without BRCA mutations.

BODY

a.   Cells and PARP1 inhibitors

The HCC1937 (BRCA1-mutated) tumor cell line is derived from a type of human ductal carcinoma bearing a BRCA1 5382insC mutation in one allele and a deletion of the second allele [23, 24]. The BRCA1 protein in HCC1937 lacks the BRCA1 C terminus, and extracts show very low levels of BRCA1 protein. The cells were stably transfected with either the wild-type BRCA1

expressing vector or the null vector so that two cell lines (BRCA1 wild-type and null-type) were established.

We have chosen olaparib (AZD2281) as the PARP1 inhibitor because it has been found to have monotherapeutic activity against tumor cells harboring BRCA1 or BRCA2 mutations. We tested the olaparib-induced cancer cell death using MTS assay [25] (Figure 1). BRCA1 wild-type cells showed resistance to 10 µM of olaparib and were slightly responsive to 25 µM of olaparib, while BRCA1 null-type cells were highly sensitive to olaparib. This set of data is consistent with the previous observation that olaparib alone has a cytotoxic effect on cancer cells.
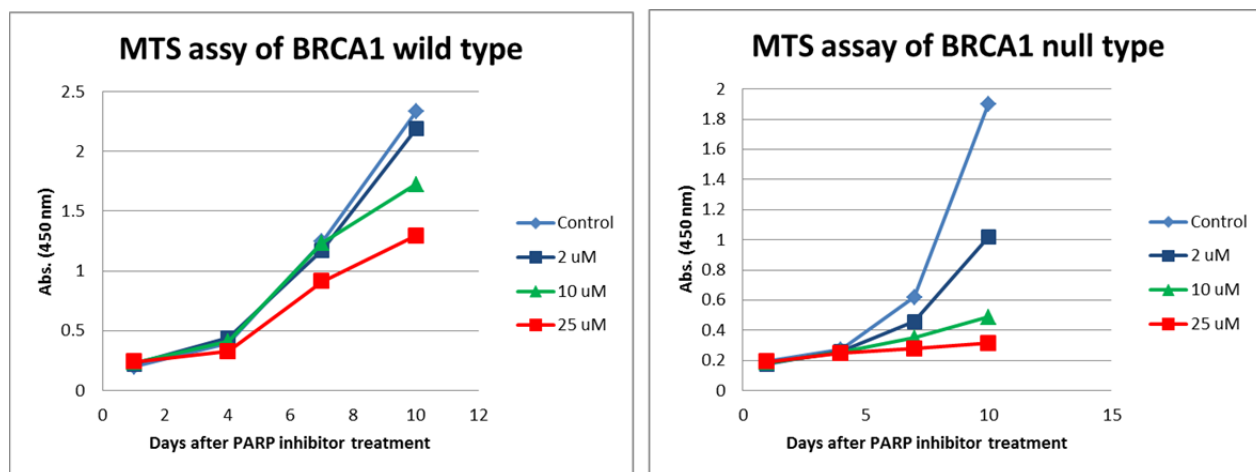

Figure 1. BRCA1 wild- and null-type cells were treated with different concentration of olaparib (2, 10, and 25 µM). Media were changed every 2 days with inhibitors. Cytotoxicity was measured by MTS assay at day 0, 4,7,10

b.   Cell Array

BRCA1 wild and null-type cells were transduced with TF activity reporting lentivirus via spinoculation [26]. Transduced cells were then be seeded on a 384-well plate with at least 4 replicates of each condition. Each well received cells that were transduced with a distinct TF reporter construct. Two days after cell seeding, luciferase activity was measured by live cell bioluminescence imaging. The 2-day period is sufficient time for ensuring lentiviral gene expression. After changing media, PARP1 inhibitors (10 µM) were added to the culture and the luciferase activity was measured every 2 days. TA lentivirus, which is composed of only minimal promoter, was used as a control. In a preliminary result, the luciferase activity from TA lentivirus transduced cells highly correlates to cell number over time. Thus, all other TF activity was normalized with respect to TA activity and represented by TF/TA ratio. As the initial experiment, we prepared and analyzed one set of cell array to monitor the activities of 34 transcription factors during treatment of the PARP1 inhibitor, olaparib, in order to identify any candidate TFs which may be responsible for inhibitor-induced cancer cell death. Two representative TF profiling graphs are shown in Figure 4 highlighting the two distinct events that the TF/TA activity increases or decreases. In case of VDR activity (top), olaparib treatment showed no effect on both BRCA1 wild and null-type cells. Of the 34 tested TFs, 19 showed similar patterns in their respective VDR activity profile. Therefore we suppose these factors are not necessarily related to the PARP1-induced cancer cell death. An example of a TF profiling pattern that shows a difference between inhibitor-treated and untreated cells or BRCA1 wild-type versus null-type cells is the AP2 activity profile (bottom). Olaparib-treated BRCA1 wild-type cells (red) showed initial increased activity in comparison to untreated cells (black). In BRCA null-type cells, AP2

activity initially shows no difference between treated and untreated cells but showed a significant difference after 8 days. From AP2 activity profile, we suppose AP2 is related with olaparib-induced BRCA1 null type cell death.
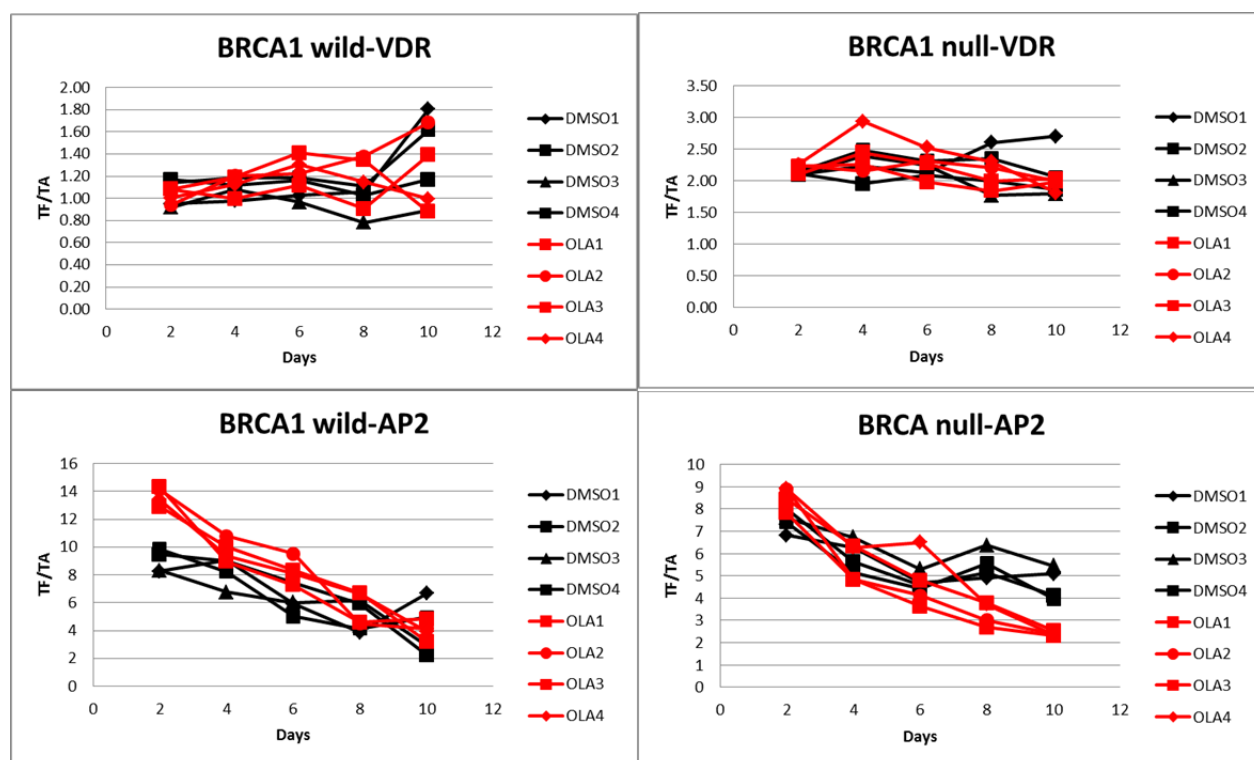

Figure 2. Representative TF activity profile of VDR and AP2 in BRCA1 wild or null type cells after treatment of 10 µM of olaparib. Each activity from four replicates were plotted with red (olaparib) or black (empty vehicle, DMSO) at each time point. All values were normalized by TF and represented by the ratio of TF/TA

As shown in table 1, 15 TFs were selected based on their increase or decrease pattern. In most cases, BRCA null-type cells showed decreased TF activity over time while BRCA wild-type cells showed increased or unchanged TF activity by olaparib treatment. It was noted that MEF1 activity decreased in both cell types, suggesting that while MEF1 is decreased by PARP1 inhibitors, it might not be related with inhibitor-induced cell death.

Ongoing studies are confirming the results of the array using independently repeated experiments. Results will also be confirmed by traditional methods such as western blotting with antibodies against transcription factors or their activated proteins. Based on the information of the TF responsible for PARP1 inhibitor-induced cancer cell death, STAT3 is currently considered as one of the transcription factors to be investigated further because STAT3 activity showed no difference between inhibitor treated/untreated-BRCA1 wild type cells but showed a significant difference in BRCA1 null type cells after 8 days. Thus, STAT3 specific inhibitors such as S3I-201[27] will be co-treated with/without PARP1 inhibitor in BRCA1 wild and null type cell.

Days	AP2 BRCA wild	AP2 BRCA null	AP3 BRCA wild	AP3 BRCA null	AR BRCA wild	AR BRCA null	CRE BRCA wild	CRE BRCA null	RXR BRCA wild	RXR BRCA null
2	1.52	1.14	1.39	0.98	1.78	1.07	1.05	1.14	1.35	1.00
4	1.18	0.94	1.11	0.70	1.07	0.99	1.17	0.90	1.17	0.92
6	1.20	1.00	0.94	0.65	1.04	0.86	1.23	0.75	1.08	0.85
8	1.12	0.60	0.99	0.64	1.14	0.79	1.39	0.71	1.15	0.76
10	0.96	0.51	1.04	0.63	1.20	1.11	0.96	0.59	0.88	0.85

Days	STAT4 BRCA wild	STAT4 BRCA null	IRF1 BRCA wild	IRF1 BRCA null	ISRE BRCA wild	ISRE BRCA null	GR BRCA wild	GR BRCA null	STAT3 BRCA wild	STAT3 BRCA null
2	1.13	1.20	1.25	1.06	1.12	1.04	0.90	0.89	1.27	0.91
4	0.99	0.93	1.13	0.94	1.07	0.82	1.05	0.83	1.13	0.93
6	1.39	0.90	1.13	0.96	1.26	0.93	1.14	0.93	1.17	0.90
8	0.78	0.90	1.38	0.87	1.28	0.69	1.36	0.90	1.33	0.66
10	0.95	0.72	1.29	0.79	0.96	0.68	0.90	0.76	1.06	0.59

Days	STAT1 BRCA wild	STAT1 BRCA null	PR BRCA wild	PR BRCA null	HIF1 BRCA wild	HIF1 BRCA null	MEF1 BRCA wild	MEF1 BRCA null	MEF3 BRCA wild	MEF3 BRCA null
2	0.96	0.98	1.11	0.81	1.00	0.97	0.73	0.97	1.09	1.01
4	1.07	1.11	1.15	0.96	0.98	0.97	0.84	0.98	1.07	0.97
6	1.25	1.07	1.20	0.80	1.12	1.05	0.67	0.94	1.17	0.86
8	1.47	1.11	1.27	0.69	1.32	0.94	0.55	0.72	1.34	0.72
10	1.30	1.12	1.55	0.99	1.16	0.68	0.64	0.59	1.13	0.62

Table 1. Ratio of transcription factor activity of inhibitor treated/untreated cells at different time points. Higher value than 1 indicates increased TF activity by inhibitor treatment. More than 20% increased and decreased TF activity values were highlighted with yellow and green, respectively. 19 out of all tested 34 TF activity profiles showed no change in both BRCA wild and null type cells.

c.   Development of PARP-1 inhibitor resistant clones from BRCA1 null-type cells

We have attempted to develop PARP1 resistant clones from BRCA1 null-type cells, which are sensitive to PARP1 inhibitor (Figure 3). PARP1 resistant clones will be established by means of either short term (1 day) exposure to high concentrations (25 µM) of PARP1 inhibitor and subsequent 10 µM (condition 1) or long term exposure to gradually increasing concentrations (0.2, 0.5, 1, 2, 5, 10 µM, 1 week in each treatment; condition 2). We failed to have clones from the condition 1, probably due to the initially high toxicity of PARP1 inhibitor. In condition 2, we had a pool of cells which can survive in the final 10 µM concentration of PARP1 inhibitor. The cells were serially diluted to single cell and enriched without PARP1 inhibitor. The drug resistances of the clones were tested by MTS assay. In preliminary result, we tested 6 clones and 5 of them showed higher drug resistance compared to parental BRCA1 null type cells (Figure 3). Interestingly, 2 of them (red) showed higher drug resistance compared to BRCA1 wild type cell (Figure 1 and Figure 3, black square). The patterns of cell survival of the other 2 clones (blue) were similar to BRCA1 wild type cells. These single cell colonies will be further identified and characterized by clonogenicity test, western blotting of BRCA1, and genomic sequencing. Based on the previous report to identify the drug resistant clones from BRCA2 mutant cells [28], we expect to obtain the resistant clones in two different subpopulations – the BRCA1 restored clones and the BRCA1 unchanged group.
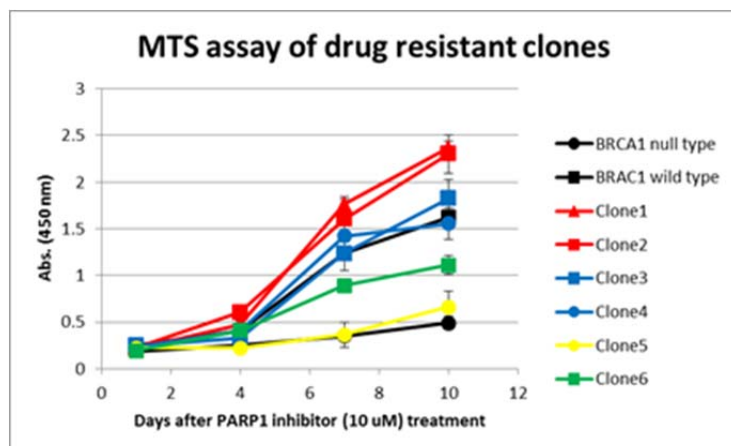
Figure 3. PARP1 inhibitor resistant clones obtained by the method of gradual increase of drug concentration were tested by MTS assay. 5 clones proved to have drug resistance compared to BRCA1 null type cells (Black circle). 2 of them (red) showed higher drug resistance compared to BRCA1 wild type cells (Black square). Data represents mean ± SD

Key Research Accomplishments:
- Measurement of growth rate for BRCA+ and BRCA- cell lines as a function of the olaparib concentration
- A total of 34 TFs were analyzed for their response to treatment with olaparib, and 15 of these 34 TFs had differential activity as a function of treatment and presence of BRCA1.
- STAT3 was selected for further analysis because STAT3 activity showed no difference between inhibitor treated/untreated-BRCA1 wild type cells, yet had a significant difference in activity for BRCA1 null type cells after 8 days. Thus, STAT3 specific inhibitors such as S3I-201[27] will be co-treated with/without PARP1 inhibitor in BRCA1 wild and null type cell.
- Five PARP-1 resistant clones were established from BRCA1 null type cells and characterized by its responsiveness to PARP1 inhibitor.

Reportable Outcomes
Presentation:

1) Michael S. Weiss, Beatriz Peñalver Bernabé, Abigail D. Bellis, Linda J. Broadbelt, Jacqueline S. Jeruss, Vincent L. Cryns, Lonnie D. Shea, "Large scale, dynamic quantification of transcription factor activity in breast cancer cells." DoD Era of Hope Conference, August, 2011

Funding applications: American Cancer Society, Illinois Division. "Basic Research for Research Assistant Professors" program. PI: Seungjin Shin. Title: Investigation of mechanism of PARP1 inhibitor induced cell death. Proposed project period: 6/1/2012 - 5/31/2013.

Conclusion: These results demonstrate the mechanism of action for the PARP inhibitor olapirib. This mechanistic understanding can be applied to understand why some patients may respond to olapirib, while others are resistant. The technology to quantify activity of numerous TFs has identified a TF that has differential activity in the presence and absence of BRCA1. Thus, supplementing olapirib with additional factors that impact the activity of this TF may enhance the efficacy of this compound.

Personnel: The following people received pay from this research effort: Jacqueline Jeruss (Co-PI), Vincent Cryns (Co-PI), Seungjin Shin (Postdoc/Research Professor), and Stanley Weng (Undergraduate Researcher). PI Lonnie Shea's effort was contributed rather than charged to the project.

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